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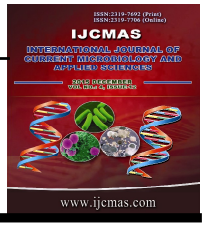

Original Research Article

Detection of ESBL Producing Gram Negative Uropathogens and their Antibiotic Resistance Pattern from a Tertiary Care Centre, Bengaluru, India

S.A. Lakshminarayana* and S. Sangeetha

Department of Microbiology, Rajarajeswari Medical College and Hospital, Bengaluru, India
**Corresponding author*

Keywords

Extended Spectrum β-Lactamase, Urinary tract infection, *E. coli*

A B S T R A C T

The Extended Spectrum β-Lactamase (ESBL) producing bacteria are increasingly causing urinary tract infections (UTIs) both in hospitalized and outpatients. Despite the widespread availability of antimicrobial agents UTI has become difficult to treat empirically because of appearance of pathogens with increasing resistance to antimicrobial agents. To determine the ESBL producing gram negative uropathogens and their antibiotic resistance pattern. This study was conducted in the department of microbiology, RajaRajeswari Medical College & Hospital, Bengaluru, over a period of 1 year. During this period around 5039 urine samples were received. Standard microbiological techniques were used to isolate and identify the organisms and to determine the antibiotic resistance pattern. A total of 793 aerobic bacterial isolates were obtained from 5039 urine samples, which comprised of 689 GNB. *E. coli* was the most predominant bacteria 65.93% followed by *Klebsiella pneumoniae* 15.41%. 28.63% (195/681) of GNB were ESBL producers. Predominant ESBL producers were *E. coli* 35.18% followed by *Citrobacter spp.* 30%, *K. pneumoniae* 21.90%. ESBL production was more prevalent in *E. coli* followed by *K. pneumoniae*. Screening and monitoring of ESBL production and antimicrobial susceptibility testing are necessary to avoid treatment failure in patients with UTI.

Introduction

Urinary tract infection (UTI) includes the infection of urethra, bladder, ureters, and kidneys, which comprise the urinary tract. Gram negative bacteria were the most common organism to cause UTIs which contribute to about 80-85%, as reported by Hussain et al. Among these, *E.coli* and *K.pneumoniae* were most frequently found. Females are highly susceptible to UTIs due to the structure and the position of the urethra and it is also caused during the time of sexual intercourse and pregnancy (Kolawale, *et al*., 2009).

Among the Gram negative pathogens, β-lactamase production remains the most important contributing factor to β-lactam resistance. The β-lactam antibiotics like penicillin have a β-lactam ring which can be hydrolyzed by β- lactamases resulting in ineffective compound (Bush K and Mobashery S). Extended spectrum β-

lactamases (ESBLs) were first described in 1983. They are able to hydrolyse oxyimino-cephalosporins (for example, cefotaxime, ceftazidime and ceftriaxone) and monobactams (for example, aztreonam), but not cephamycins or carbapenems (Patricia et al and Bush K).

The ESBL producing bacteria are increasingly causing urinary tract infections (UTI) both in hospitalized and outpatients. This makes therapy of UTI difficult with prolonged hospital stay, increased morbidity, mortality and health care costs (Mehrgan H and Rahbar M). It promotes greater use of expensive broad spectrum antibiotics, such as Beta-lactum/Beta-lactaum inhibitors and carbapenems.

The aim of our study was to evaluate the antimicrobial resistance of ESBL and non-ESBL producing gram negative organisms in patients attending a tertiary care hospital.

Materials and Methods

The study was conducted in the department of microbiology, RajaRajeswari Medical College & Hospital from August 2015 to September 2015. Urine samples were received from various outpatient departments (OPDs) and inpatient departments (IPDs). Clean catch, mid-stream urine samples were collected in sterile universal containers. Urine samples were processed within 2 hours of collection and in case of delay, the samples were refrigerated at 2-8^{O}C for up to 6 hours.

The samples were inoculated onto 5% sheep blood agar, MacConkeys agar and Cysteine lactose electrolyte deficient (CLED) media by the semi-quantitative plating method using a calibrated loop delivering 0.001ml of urine. The plates were incubated at 37 ^{0} C for 24-48 hours. Interpretation of cultures and identification of gram negative isolates was done as per standard protocol (Forbes BA et al).

Antimicrobial Susceptibility Testing

Isolates were screened initially using Kirby-Bauer method on Mueller Hinton as per CLSI guidelines (CLSI 2014). All ESBL producing isolates were confirmed by using combined disc test (CDT) with antibiotic discs containing ceftazidime (30μg) and cefotaxime (30μg) either alone or in combination with clavulanic acid (10μg). An isolate was considered to be an ESBL producer if the zone of inhibition around the ceftazidime/clavulanic acid or cefotaxime/clavulanic acid disc was > 5 mm than the zone around the ceftazidime or cefotaxime disc alone.

Susceptibility testing to other antibiotics was performed by disk diffusion methods as recommended by clinical laboratory standard institute (CLSI).

Statistical Analysis

Data was entered into a computerized Excel (Microsoft Excel 2009) spread sheet, and subsequently it was analyzed using SPSS (trial version 20) software. Descriptive statistics (means and percentages) were used wherever necessary.

Results and Discussion

A total of 793 isolates were obtained from 5039 urine samples, which comprised of 689 Gram negative isolates, 112 Gram positive cocci of which 76 were *coagulase negative Staphylococcus* (CONS), 10 were *Staphylococcus aureus* and 21 were *Entreococcus spp.*

Only 5 *Candida spp.* were isolated. Culture positivity in male and female patients was 40.09% (318/793) and 59.91% (475/793) respectively. 46.84% of outpatients (OPDs) and 53.15% of inpatients (IPDs) urine samples were culture positive.

Among the GNB, *E. coli* was the most predominant bacteria 65.93% followed by *Klebsiella pneumoniae* 15.41%, *K. oxytoca* (5.43%), *Pseudomonas spp.* (3.52%), *P. mirabilis* (2.93%) and *Non-fermenting gram negative bacilli* (2.49%) (Table 1).

Overall, 28.63% (195/681) of GNB were ESBL producers. Predominant ESBL producers were *E. coli* 35.18% followed by *Citrobacter spp.* 30%, *K. pneumoniae* 21.90%, *Enterobacter spp.* 16.66% and *K oxytoca* 16.21% (Table 1).

ESBL production was more often associated with infection in patients with more than 35 years of age, in female 53.33% (104/195) and in patients 81.02% (158/195) (Table 3 & 4). The antibiotic resistance pattern of ESBL producers and Non-ESBl producers were detailed in the table 4.

ESBL are being increasingly described worldwide. Detection of ESBL producing organisms is a challenge for the laboratories. Ours is a tertiary care hospital with all the medical departments including the super speciality departments. We carried out this study mainly to know the percentage of ESBL producing organisms in urine samples in our hospital and also to know the susceptibility pattern of ESBL producing organisms.

In our study, 681 (13.51%) gram negative organisms were obtained from total urine samples processed. *E. coli* was the most common isolate 65.93% followed by *K. pneumonia* (15.41%), *K. oxytoca* (5.43%) and P. mirabilis (2.93%), whereas the incidence of other Enterobacteriaceae is low. Our findings coincides with the studies of Khan IU et al and Amin M et al which showed 61.3% and 59% *E. coli* respectively.

The isolation of *Pseudomonas spp* was (3.52%) and NFGNB was (2.49%) which is similar to the other studies (Singhal A et al, Amin M et al).

Several studies have reported the incidence of ESBL producers among uropathogens ranging from 8.9% to 71.5% (Kader AA et al and Nachimuthu R et al). In the present study, 28.63% (195) were ESBL producers, which coincide with study of Asha B patil et al (27.86%).

The predominant ESBL positive isolate in our study was *E. coli* (35.18%) followed by *Citrobacter spp.*(30%), *K. Pneumoniae* (21.9%), *Enterobacter spp.*(16.66%), *K. oxytoca* (16.21%). Ritu A et al, reported E. coli (40%) and Citrobacter spp. (44.44%) as the predominant ESBL producer, our study correlates with this study.

We noticed, ESBL production was more often associated with infection in patients with above 35 years and female patients 53.33% compared to male patients 46.67%. About 81.02% of IPD patients and 18.97% of OPD patients showed ESBL positivity. Duration of catheterisation for more than 7 days, long hospital stay, diabetes mellitus and prior antibiotic therapy with irrational drugs found to be associated with higher rates of ESBL production.

On comparison of resistance pattern rates between ESBL producers and non-ESBL producers (Table 4), both showed high and comparable resistance to ampicillin (100% and 83.3%), cephlalexin (100% and 88.9%), cefuroxime (100% and 88.6%),

amoxicillin/clavulanic acid (100% and 73.4%),ceftriaxone (100% and 72%), ceftazidime (100% and 67.4%), cefepime (97.43% and 41.7%) respectively.

Table.1 Distribution of Gram Negative Bacilli and ESBL Producing GNB from Urine Samples

Isolate	Total No. GNB(n=681)	ESBL producing GNB
E. coli	449 (65.93%)	158 (35.18%)
K. pneumoniae	105 (15.41%)	23 (21.90%)
K. oxytoca	37 (5.43%)	6 (16.21%)
P. mirabilis	20 (2.93%)	2 (10%)
P. vulgaris	9 (1.32%)	1 (11.11%)
Enterobacter spp.	6 (0.88%)	1 (16.66%)
Citrobacter spp.	10 (1.46%)	3 (30%)
Morganella spp	4 (0.58%)	00
Providencia spp	2 (0.29%)	00
Pseudomonas spp.	24 (3.52%)	1 (4.16%)
Non –fermenting GNB	17 (2.49%)	00
Total	**681**	**195 (28.63%)**

Table.2 Distribution of ESBL Producers among Male and Female

	Male	Female
ESBL Positive (n=195)	91 (46.67%)	104 (53.33%)
ESBL Negative (n=486)	182 (37.44%)	304 (62.55%)

Table.3 Distribution of ESBL Producers among out Patients and Inpatients

	OPD	IPD
ESBL Positive (n=195)	37 (18.97%)	158 (81.02%)
ESBL Negative (n=486)	282 (58.02%)	204 (41.97%)

Table.4 Percentage of Resistance to Antibiotics among ESBL Producers and Non-ESBL Producers

Antibiotics	**ESBL producers (n=195)**	**Non-ESBL producers (n=486)**
Ampicillin (30µg)	100%	83.3%
Amikacin (30µg)	11.7%	9.5%
Amoxicillin/clavulanic acid (20/10µg)	100%	73.4%
Cephalexin (30µg)	100%	88.9%
Cefuroxime (30µg)	100%	88.6%
Cefotaxime (30µg)	100%	72%
Ceftazidime (30µg)	100%	67.4%
Cefepime (30µg)	97.43%	41.7%
Cefoxitin (30µg)	50%	60%
Piperacillin-tazobactum (100/10µg)	8.8%	6.5%
Gentamicin (10µg)	55.7%	37.8%
Tobramycin (10µg)	8.1%	6.3%
Norfloxacin (10µg)	85.1 %	67.2%
Ciprofloxacin (5µg)	84.9%	65.6%
Ofloxacin (5µg)	50%	33.3%
Cotrimoxazole (1.25/23.75µg)	68.6%	58.3%
Nitrofurantoin (300µg)	15.4%	10.8%
Aztreonam (30µg)	73.1%	41.7%
Tetracycline (30µg)	10.8%	9.8%
Meropenem (10µg)	7.9%	4.3%
Imipenem (10µg)	1.2%	1%
Colistin (10µg)	00	00
Tigecycline (15µg)	00	00

However, both ESBL producers and non-ESBL producers showed relatively less resistance to amikacin (11.7% and 9.5%), tobramycin (8.1% and 6.3%), tetracycline (10.8% and 9.8%), nitrofurantoin (15.4% and 10.8%) respectively. Among the fluoroquinolones tested ESBL producers were more resistant to norfloxacin (85.1%) followed by ciprofloxacin (84.9%) and ofloxacon (50%).

ESBL producers were more sensitive to imipenem (98.8%) followed by meropenem (92.1%), piperacillin-tazobactum (91.2%). Syed MA et al reported about 1.5% imipenem, 10.8% amikacin, 86.2% ciprofloxacin, 81.5% norfloxacin resistance among ESBL producers, which is comparable with present study. Colistin and tigecyclin showed 100% sensitivity by all gram negative organisms, but these drugs are kept as reserve, should be used judicially.

In conclusion, our study showed that ESBL production was more prevalent in *E. coli* followed by *K. pneumoniae*. Most of the ESBL and Non ESBL producing isolates were resistant to common antibiotics used for the treatment of Urinary Tract Infection (UTIs). This study is important for implementation of using proper antibiotics and also to take steps for reducing multidrug resistance of UTIs causing bacterial isolates. Continuous monitoring of surveillance studies needs to be performed in our hospital

to proper treatment and control of antibacterial resistance, especially Extended Spectrum β-Lactam antibiotic resistance.

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